INTRODUCTION

- Mometasone furoate is a potent synthetic corticosteroid with minimal local irritative effects when administered topically (dermatitis) or via inhalation (allergy, asthma).
- Maximum plasma concentrations near 50 pg/mL are expected following inhalation of 100 – 400 µg, and a sensitive method is required to measure an adequate number of data points for calculating pharmacokinetic parameters.
- A post-extraction derivatization procedure coupled with a dual-column LC-MS/MS setup provided sufficient sensitivity to validate an LLOQ of 0.25 pg/mL.

Figure 1. Mometasone furoate and hydracine reaction products

The target LLOQ of 0.25 pg/mL was challenging to achieve as the neutral steroid tends to form adducts in positive ESI and the signal is diluted by the presence of 2 chlorine atoms. Derivatization with several hydrazine compounds or dansyl chloride was investigated to increase and stabilize the response. Two isomeric products were often observed with reversed phase chromatography.

Figure 2. Reversed phase chromatography of derivatised mometasone furoate.

The reaction products presented themselves as a single peak using cation exchange chromatography with moderate retention.

Figure 3. Mometasone furoate derivative SCX chromatography

SAMPLE PREPARATION

Several solvents were tested to find the highest retention of the chlorinated chloride at alkaline pH which was chosen to provide the cleanest baseline and adequate recovery from 1.0 mL of EDTA plasma prior to derivatization.

Figure 4. Post column infusion experiment, mometasone furoate retention time ca. 1.6 minutes under typical (not final) SCX conditions.

INSTRUMENTATION

A dual-column back-flush method was adopted. In this approach, 2 columns are used under isocratic conditions with one being rinsed offline in the reverse direction while the analysis column is eluting to the mass spec. Alternating column via a valve switch eliminates downgradient collision of suppressive compounds.

Mobile Phase: Acetonitrile:Ammonium formate:15 mM ammonium formate, pH 2.5; Analytical Flow Rate 1.0 mL/min

• Column: SCX 3x50 mm 5µm, 50°C

• Mobile Phase: Acetonitrile:Methanol:15 mM ammonium formate, pH 2.5; Analytical Flow Rate 0.9 mL/min, Back-flush Flow Rate 1.0 mL/min

• Determination: Electrospray ionization (ESI) data was acquired by multiple reaction-monitoring (MRM) in positive mode on an AB SCIEX 5500 Q-TRAP mass spectrometer. Acquisition time 2.0 minutes.

RESULTS

- The validated analytical range was 0.25 to 25.0 pg/mL of Mometasone furoate using 1.0 mL of EDTA plasma samples.
- The inter-batch precision (% C.V.) and accuracy (% Bias) of quality control samples is shown in Table 1.
- The extraction recovery of Mometasone furoate was 87 ± 50%
- Selectivity and matrix effect data were acceptable for healthy and hemolysed human EDTA plasma.
- Mometasone furoate was stable in human EDTA plasma through 5 freeze/thaw cycles, inclusive of 53 hours at ambient temperature under white lighting and up to 57 days when stored at -20°C.
- Derivatized mometasone furoate integrity was maintained up to 78 hrs post extraction.

Figure 5. Dual column back-flush LC-MS/MS design.

Table 1. Validation Interday Reproducibility

<table>
<thead>
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<th>QC Level</th>
<th>Precision % C.V.</th>
<th>Accuracy % Bias</th>
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</thead>
<tbody>
<tr>
<td>Low</td>
<td>16.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Low</td>
<td>7.6</td>
<td>2.8</td>
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<td>High</td>
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</tbody>
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Figure 6. Final conditions for mometasone furoate derivative.

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